temperature. The first antibody is added at a pre-determined dilution in TBS/BSA for 1 h at room temperature. Three washes with TBS followed, and cells are briefly reblocked for 15 min at room temperature. Incubation with goat anti-mouse conjugated alkaline phosphatase (Bio-Rad) diluted 1:1000 in TBS/BSA is carried out for 1 h at room temperature. The cells are washed three times with TBS and a colorimetric alkaline phosphatase substrate is added. When the adequate color change is reached, 100-μl samples are taken for colorimetric readings.

mRNA Expression

[0459] Expression of mRNA is quantitated by RT-PCR using TaqMan® technology. Total RNA is isolated from cancer model cell lines using the RNEasy 96 kit (Qiagen) per manufacturer's instructions and included DNase treatment. Target transcript sequences are identified for the differentially expressed peptides by searching the BlastP database. TaqMan assays (PCR primer/probe set) specific for those transcripts are identified by searching the Celera Discovery System™ (CDS) database. The assays are designed to span exon-exon borders and do not amplify genomic DNA. The TaqMan primers and probe sequences are as designed by Applied Biosystems (AB) as part of the Assays on Demand™ product line or by custom design through the AB Assays by Design^{SM} service. RT-PCR is accomplished using AmpliTaqGold and MultiScribe reverse transcriptase in the One Step RT-PCR Master Mix reagent kit (AB) according to the manufacturers instructions. Probe and primer concentrations are 900 nM and 250 nM, respectively, in a 25 μl reaction. For each experiment, a master mix of the above components is made and aliquoted into each optical reaction well. 5 ul of total RNA is the template. Each sample is assayed in triplicate. Quantitative RT-PCR is performed using the ABI Prism® 7900HT Sequence Detection System (SDS). Cycling parameters follow: 48° C. for 30 min. for one cycle; 95° C. for 10 min for one cycle; 95° C. for 15 sec, 60° C. for 1 min. for 40 cycles.

[0460] The SDS software calculates the threshold cycle (C_T) for each reaction, and C_T values are used to quantitate the relative amount of starting template in the reaction. The C_T values for each set of three reactions are averaged for all subsequent calculations.

[0461] Total RNA is quantitated by using RiboGreen RNA Quantitation Kit according to manufacturer's instructions and the % mRNA expression is calculated using total RNA for normalization. % knockdown is then calculated relative to the no addition control.

13. In Vivo Studies by Using Antibodies

[0462] Treatment of Kidney Cancer Cells with Monoclonal Antibodies.

[0463] Kidney cancer cells are seeded at a density of 4×10^4 cells per well in 96-well microtiter plates and allowed to adhere for 2 hours. The cells are then treated with different concentrations of anti-KCAT monoclonal antibody (Mab) or irrelevant isotype matched (anti-rHuIFN-. gamma. Mab) at 0.05, 0.5 or 5.0 mug/ml. After a 72 hour incubation, the cell monolayers are stained with crystal violet dye for determination of relative percent viability (RPV) compared to control (untreated) cells. Each treatment group consists of replicates. Cell growth inhibition is monitored.

Treatment of NIH 3T3 Cells Overexpression KCAT Protein with Monoclonal Antibodies.

[0464] NIH 3T3 expressing KCAT protein are treated with different concentrations of anti-KCAT MAbs. Cell growth inhibition is monitored.

In Vivo Treatment of NIH 3T3 Cells Overexpressing KCAT with Anti-KCAT Monoclonal Antibodies.

[0465] NIH 3T3 cells transfected with either a KCAT expression plasmid or the neo-DHFR vector are injected into nu/nu (athymic) mice subcutaneously at a dose of 10^6 cells in 0.1 ml of phosphate-buffered saline. On days 0, 1, 5 and every 4 days thereafter, 100 mug (0.1 ml in PBS) of either an irrelevant or anti-KCAT monoclonal antibody of the IG2A subclass is injected intraperitoneally. Tumor occurrence and size are monitored for 1 month period of treatment.

[0466] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention, which are obvious to those skilled in the field of molecular biology or related fields, are intended to be within the scope of the following claims.

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20210277073A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. An isolated protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:1-2736 and 5165-6044.

2. A composition comprising the protein of claim **1** and a pharmaceutically acceptable carrier.

3. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) SEQ ID NOS:2737-5164;

b) nucleotide sequences that encode a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:1-2736 and 5165-6044; and